Control of Gluconeogenesis and of Enzymes of Glycogen Metabolism in Isolated Rat Hepatocytes

A PARALLEL STUDY OF THE EFFECT OF PHENYLEPHRINE AND OF GLUCAGON

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Hepatocytes isolated from the livers of fed rats were used for a comparative study of the effects of phenylephrine, vasopressin and glucagon on gluconeogenesis and on enzymes of glycogen metabolism. When hepatocytes were incubated in the presence of Ca^{2+} , phenylephrine stimulated gluconeogenesis from pyruvate less than did glucagon, but, in contrast with this hormone, it did not affect the activities of protein kinase and pyruvate kinase, nor the concentration of phosphoenolpyruvate, and it did not decrease the release of $^3\text{H}_2\text{O}$ from $[6\text{-}^3\text{H}]\text{glucose}$. The effects of vasopressin were similar to those of phenylephrine. Gluconeogenesis from fructose was also stimulated by phenylephrine and, more markedly, by glucagon at the expense of the conversion of fructose into lactate. Insulin was able to antagonize the stimulatory effect of phenylephrine on gluconeogenesis from pyruvate. When Ca^{2+} was removed from the incubation medium, phenylephrine still stimulated gluconeogenesis from pyruvate, but it also caused an activation of protein kinase and an inactivation of pyruvate kinase; accordingly, the concentration of phosphoenolpyruvate was increased, and, in contrast, vasopressin had no effect on all these parameters. The property of phenylephrine to cause the activation of glycogen phosphorylase was decreased by glucose or by the absence of Ca^{2+} ; it was abolished when these two conditions were combined. Glycogen synthase was inactivated by phenylephrine in the presence or the absence of Ca^{2+} , although presumably by different mechanisms.

Phenylephrine, an α -adrenergic agonist (Tolbert *et al.*, 1973; Exton & Harper, 1975), and vasopressin (Hems & Whitton, 1973) are known to cause a stimulation of gluconeogenesis in isolated liver preparations, which reaches 30-50% of that by glucagon, but is characterized by an absence of modifications in the concentration of cyclic AMP and activation of protein kinase (Sherline *et al.*, 1972; Kirk & Hems, 1974; Keppens & De Wulf, 1975; Cherrington *et al.*, 1976). The same agonists also stimulate glycogen breakdown [for reviews see Hers (1976) and Hems (1977)] and their effect is currently believed to be mediated by ionic changes, presumably an increase in Ca^{2+} concentration (Stubbs *et al.*, 1976; Assimacopoulos-Jeannet *et al.*, 1977; Keppens *et al.*, 1977; van de Werve *et al.*, 1977a). The present work is an investigation of the mechanism by which phenylephrine and vasopressin stimulate gluconeogenesis. Particular attention is paid to the activity of pyruvate kinase, since an inactivation of the latter enzyme parallels the stimulation of gluconeogenesis

by glucagon or cyclic AMP (Feliú *et al.*, 1976). A similar inactivation of pyruvate kinase by glucagon and cyclic AMP has also been reported by others (Blair *et al.*, 1976; Riou *et al.*, 1976; Van Berkel *et al.*, 1976). Phosphorylation of pyruvate kinase by the cyclic AMP-dependent protein kinase causes such an inactivation (Ljungström *et al.*, 1974) and is presently the only molecular mechanism known in the control of gluconeogenesis. Since the control of glycogen metabolism by both cyclic AMP-dependent and -independent mechanisms shows many similarities to that of gluconeogenesis, changes in the activity of phosphorylase and of glycogen synthase were measured for the sake of comparison.

Part of this work has been reported in a preliminary form (Hue & Feliú, 1978).

Materials and Methods

Chemicals

Glucose, EGTA, glucose oxidase, type I collagenase, calf thymus histone II-A, L-phenylephrine, synthetic vasopressin grade 6 (Sigma Chemical Co., St. Louis, MO, U.S.A.), bacitracin (Aldrich Chemical

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Co., Milwaukee, WI, U.S.A.), ion-exchange resin AG 1 (X8; 200–400 mesh; Bio-Rad Laboratories, Richmond, CA, U.S.A.), [γ - ^{32}P]ATP, [$\text{U-}^{14}\text{C}$]pyruvate, [$\text{U-}^{14}\text{C}$]fructose, [$6\text{-}^3\text{H}$]glucose (The Radiochemical Centre, Amersham, Bucks., U.K.), glucagon, insulin (Novo-Industri A/S, Copenhagen, Denmark) and other biochemical reagents (Sigma or Boehringer G.m.b.H., Mannheim, Germany) were purchased as indicated.

Isolation and incubation of hepatocytes

Livers of normally fed male Wistar rats weighing about 250 g were used. For the isolation of hepatocytes the general procedure of Krebs *et al.* (1974) was used with the following modifications: the liver was perfused with 300 ml of Krebs–Henseleit bicarbonate buffer (Krebs & Henseleit, 1932) without Ca^{2+} and enriched with 10 mM-glucose. The first 200 ml of the medium passing through the liver was discarded and 50 mg of collagenase suspended in a small volume of medium was added. The duration of perfusion was 15 min and the incubation of disrupted liver with the same medium lasted for only 1 min. The cell suspension was filtered through a few layers of cheesecloth and centrifuged for 1 min at 50 g. The cell pellet was washed with 60–80 ml of Krebs–Henseleit medium containing 2.5 mM- CaCl_2 and filtered again through cheesecloth. After centrifugation, cells were suspended in about 20 vol. of the same medium. When hepatocytes were incubated in the absence of Ca^{2+} , they were suspended in Krebs–Henseleit medium without Ca^{2+} , but containing 2.5 mM-EGTA.

A 2 ml portion of this cell suspension [corresponding to about 20 mg of protein as measured by the method of Lowry *et al.* (1951) with bovine serum albumin as standard] were shaken (120 strokes/min) in stoppered 20 ml vials at 37°C in the presence of 10 mM-glucose and 0.12% bacitracin. The gas phase was O_2/CO_2 (19:1, v/v). Glucagon, phenylephrine or vasopressin and radioactive precursors were added after 30 min of incubation; 5 min later, 0.1 ml samples of the cell suspension were taken and frozen in a cooling mixture (solid CO_2 in acetone) for the determination of enzyme activities. Then, 20 min after the addition of labelled precursor, 0.5 ml samples of cell suspension were taken for the determination of $^3\text{H}_2\text{O}$ or radioactive glucose formed from pyruvate or fructose.

Measurement of enzyme activities and of metabolites

Methods for homogenization of the cells and for the measurement of phosphorylase *a*, glycogen synthase *a* and pyruvate kinase have been described elsewhere (Hue *et al.*, 1975; Felíu *et al.*, 1976). The activities of phosphorylase *a* and synthase *a* were measured at 25°C. One unit of phosphorylase *a* and synthase *a* is the amount of enzyme that converts

1 μmol of substrate/min under the conditions of the assay.

The activity of pyruvate kinase was measured at 0.15 mM-phosphoenolpyruvate and is referred to as $v_{0.15}$. The change in $v_{0.15}$ was in our hands the best measurement of the interconversion of the two forms of pyruvate kinase, since these two forms have the same velocity measured at 5 mM-phosphoenolpyruvate (Felíu *et al.*, 1976, 1977).

The activity of protein kinase was measured with histone as substrate as described by van de Werve *et al.* (1977b). The activity is expressed as the activity ratio, which is the ratio of the activity in the absence divided by the activity in the presence of 5 μM -cyclic AMP after subtraction of the activity measured in the presence of the heat-stable protein inhibitor of protein kinase (Walsh *et al.*, 1971).

The phosphoenolpyruvate concentration was measured by an enzymic method (Czok & Lamprecht, 1974).

Measurement of gluconeogenesis and glycolysis

Gluconeogenesis from 2 mM- $[\text{U-}^{14}\text{C}]$ pyruvate was measured as described by Felíu *et al.* (1976), except that Dowex AG 1 (X8; Cl^- form) was used instead of a mixed-bed resin (AG 50 1; X8) to separate radioactive glucose from labelled anions.

To measure gluconeogenesis from fructose, glucose and lactate were separated from labelled fructose. The following procedure was used. The reaction was stopped by the addition of 0.5 ml of 20% (w/v) HClO_4 . After neutralization a 1 ml portion of the deproteinized extract was passed through a column (0.5 cm $\times$ 4 cm) of Dowex AG 1 (X8; Cl^- form) to remove free sugars from anions (mainly lactate), which were then eluted with 1 M- NaCl . A 1 ml portion of the 'sugar fraction' was incubated with glucose oxidase (1 mg/ml) in the presence of 25 mM-Tris/HCl, pH 8, for 3 h at 37°C with gentle shaking and then passed again through a column (0.5 cm $\times$ 4 cm) of Dowex (Cl^- form). Fructose was washed out with water; gluconate formed from glucose was retained on the column and could be eluted with 1 M- NaCl .

Glycolysis was estimated by the rate of formation of $^3\text{H}_2\text{O}$ from $[\text{6-}^3\text{H}]$ glucose (Katz & Rognstad, 1975). Separation of $^3\text{H}_2\text{O}$ water from glucose was carried out as described by Bontemps *et al.* (1978).

Radioactivity was measured with a liquid-scintillation spectrometer by using the scintillation mixture of Patterson & Greene (1965).

Results

Data concerning gluconeogenesis and enzymes of glycogen metabolism have often been obtained in the same experiments and are therefore presented together in the same Figures or Tables. However, for

the sake of clarity they are described separately in the text.

Stimulation of gluconeogenesis by phenylephrine and vasopressin with no change in the activity of pyruvate kinase in hepatocytes incubated with 2.5 mM-Ca²⁺

Fig. 1 shows that increasing concentrations of phenylephrine caused a progressive increase, up to 2-fold, in the rate of gluconeogenesis from 2 mM-

Fig. 1. Effect of various concentrations of phenylephrine on the activities of phosphorylase a and of pyruvate kinase and on the rate of gluconeogenesis of isolated hepatocytes incubated in the presence of 2.5 mM-Ca²⁺

Enzyme activities were measured in samples taken 10 min after the addition of the agonist. Values shown are means $\pm$ S.E.M. (vertical bars) for three different observations with a single batch of cells. Symbols: $\circ$, gluconeogenesis; $\bullet$, pyruvate kinase activity; $\blacksquare$, phosphorylase a activity.

[U-¹⁴C]pyruvate measured over a period of 20 min without significant change in the activity of pyruvate kinase measured at 10 min after the addition of the agonist. The activity of pyruvate kinase remained unchanged during all the experimental period of 20 min after the addition of 10 μ M-phenylephrine, whereas it was rapidly and persistently inactivated by 1 μ M-glucagon (results not shown). Fig. 2 shows that phenylephrine was also unable to modify the intensity of the glucagon effect on pyruvate kinase or the sensitivity to this last hormone; phenylephrine stimulated gluconeogenesis to an extent that was reached in the presence of a dose of glucagon that inactivated pyruvate kinase by about 30%. Insulin partially antagonized the effect of saturating concentrations of phenylephrine on gluconeogenesis and on phosphorylase activation (Table 1). The same Table shows the well-known antagonism of insulin toward the effect of suboptimal concentration of glucagon on both gluconeogenesis and pyruvate kinase activity. As shown in Table 2, vasopressin had an effect comparable with that of phenylephrine, with again no change in pyruvate kinase activity. These two agonists were unable to increase the activity of histone kinase, which was greatly activated by glucagon.

The question still remains, however, whether the actual activity of pyruvate kinase, irrespective of its phosphorylation state, could be decreased by phenylephrine or vasopressin. To evaluate changes in pyruvate kinase activity, we have measured (1) the concentration of phosphoenolpyruvate, which is expected to be elevated when pyruvate kinase is less active, and (2) the rate of release of ³H from [6-³H]-glucose. Table 2 shows that phosphoenolpyruvate

Fig. 2. Effect of various concentrations of glucagon on the activity of pyruvate kinase and on the rate of gluconeogenesis of isolated hepatocytes incubated with (●) or without (○) 10 μ M-phenylephrine. The incubation mixture contained 2.5 mM-Ca²⁺.

Table 1. Effect of glucagon and phenylephrine on the rate of gluconeogenesis and on the activities of pyruvate kinase and phosphorylase of hepatocytes incubated with or without insulin in the presence of 2.5 mM- Ca^{2+}

Insulin was added 10 min before the addition of the other hormones. Values shown are means $\pm$ S.E.M. for three observations with one batch of cells.

Additions	Gluconeogenesis (μmol of pyruvate converted into glucose/20 min per g of cells)	Pyruvate kinase activity ($v_{0.15}$ /g of cells)	Phosphorylase α (units/g of cells)
No insulin			
0.15 M-NaCl	1.10 $\pm$ 0.16	23.4 $\pm$ 1.5	3.8 $\pm$ 0.2
10 μM -Phenylephrine	2.60 $\pm$ 0.06	21.3 $\pm$ 1.6	16.6 $\pm$ 0.6
0.3 nM-Glucagon	3.07 $\pm$ 0.71	11.5 $\pm$ 2.6	13.9 $\pm$ 0.3
With 10 nM-insulin			
0.15 M-NaCl	0.85 $\pm$ 0.12	24.1 $\pm$ 0.8	3.6 $\pm$ 0.1
10 μM -Phenylephrine	1.26 $\pm$ 0.12	23.3 $\pm$ 1.5	14.9 $\pm$ 0.4
0.3 nM-Glucagon	1.13 $\pm$ 0.10	17.4 $\pm$ 0.4	6.7 $\pm$ 0.5

Fig. 3. Effect of removal of Ca^{2+} from the incubation medium on the activities of glycogen phosphorylase α (a), glycogen synthase α (b) and pyruvate kinase (c) of hepatocytes incubated with two concentrations of glucose after the addition of 0.15 M-NaCl ($\circ$), 10 μM -phenylephrine ($\bullet$) or 1 μM -glucagon ($\blacksquare$)

Cells were incubated in the presence of 2.5 mM- Ca^{2+}

concentrations were slightly, but not significantly, increased after phenylephrine and that there was no decrease in the rate of release of ^3H from [6- ^3H]-glucose; on the contrary, there was a small, but barely significant, increase after phenylephrine and vasopressin. By contrast, these two parameters were greatly modified in the presence of glucagon.

Gluconeogenesis from 1 mM-[U- ^{14}C]fructose was stimulated by phenylephrine and, more markedly, by glucagon at the expense of the conversion of fructose into lactate (Table 3).

Effect of the removal of Ca^{2+} from the incubation medium on the stimulation of gluconeogenesis by phenylephrine and vasopressin (Table 2)

The removal of Ca^{2+} from the incubation medium decreased the basal rate of gluconeogenesis without changing the activity of pyruvate kinase. The most striking observation was that phenylephrine now caused an activation of histone kinase accompanied by an inactivation of pyruvate kinase. Accordingly, the concentration of phosphoenolpyruvate was increased and the release of ^3H from [6- ^3H]glucose was decreased. By contrast, the stimulation of gluconeogenesis by vasopressin was completely abolished and none of the other parameters was affected by this agent.

The removal of Ca^{2+} did not greatly modify the effect of glucagon on the inactivation of pyruvate kinase although, as described by Pilkis *et al.* (1975), the stimulation of gluconeogenesis was impaired.

Effect of phenylephrine, vasopressin and glucagon on the enzymes of glycogen metabolism

In hepatocytes incubated in the presence of Ca^{2+} , glycogen phosphorylase was activated by glucagon, phenylephrine and vasopressin (Figs. 1 and 3, Tables

or 2.5 mM-EGTA with 10 or 50 mM-glucose as indicated.

Table 2. Effects of phenylephrine, glucagon and vasopressin on the rate of gluconeogenesis, on the activities of pyruvate kinase, phosphorylase, histone kinase and on the pyruvate kinase flux in hepatocytes incubated with or without Ca^{2+}

Values shown are means $\pm$ s.e.m. for the numbers of cell preparations in parentheses. Values that are statistically different as calculated by the paired *t* test are indicated as * $P < 0.05$ and ** $0.1 < P < 0.05$. Abbreviation: ND, not determined.

	Gluconeogenesis (μmol of pyruvate converted into glucose/20 min per g of cells)	Pyruvate kinase ($v_{0.15}$ /g of cells)	Phosphorylase α (units/g of cells)	Histone kinase (activity ratio)	Release of ^3H from [6- ^3H]glucose (μmol of glucose/20 min per g of cells)	[Phosphoenol- pyruvate] (mM)
With 2.5 mM- Ca^{2+}						
Control	1.6 $\pm$ 0.1 (10)	13.8 $\pm$ 1.0 (11)	3.7 $\pm$ 0.8 (11)	0.13 $\pm$ 0.02 (3)	1.7 $\pm$ 0.1 (3)	0.22 $\pm$ 0.02 (3)
10 μM -Phenylephrine	2.6 $\pm$ 0.2 (9)*	13.4 $\pm$ 1.0 (11)	13.6 $\pm$ 1.1 (11)*	0.09 $\pm$ 0.02 (3)	2.0 $\pm$ 0.2 (3)**	0.27 $\pm$ 0.04 (3)
1 μM -Glucagon	4.3 $\pm$ 0.3 (8)*	3.8 $\pm$ 0.5 (10)*	18.2 $\pm$ 1.3 (10)*	0.75 $\pm$ 0.04 (3)*	0.6 $\pm$ 0.1 (3)*	0.54 $\pm$ 0.12 (3)*
10 nM-Vasopressin	2.1 $\pm$ 0.2 (3)*	14.3 $\pm$ 2.8 (3)	12.0 $\pm$ 2.3 (3)*	0.11 (2)	2.6 (2)	ND
Without Ca^{2+} + 2.5 mM-EGTA						
Control	1.1 $\pm$ 0.3 (3)	14.8 $\pm$ 2.4 (6)	1.9 $\pm$ 0.3 (6)	0.12 $\pm$ 0.02 (3)	2.1 $\pm$ 0.2 (3)	0.19 (2)
10 μM -Phenylephrine	2.2 $\pm$ 0.2 (3)*	7.4 $\pm$ 1.5 (6)*	8.6 $\pm$ 1.5 (6)*	0.30 $\pm$ 0.05 (3)*	1.1 $\pm$ 0.2 (3)*	0.29 (2)
1 μM -Glucagon	2.3 $\pm$ 0.2 (3)*	3.8 $\pm$ 1.0 (6)*	16.9 $\pm$ 1.9 (6)*	0.82 $\pm$ 0.03 (3)*	0.8 $\pm$ 0.1 (3)*	0.44 (2)
10 nM-Vasopressin	0.8 (2)	13.9 $\pm$ 1.2 (3)	2.2 $\pm$ 0.6 (3)	0.03 (2)	2.0 (2)	ND

1 and 2). The extent of activation by the two last effectors was smaller than that brought about by glucagon. Fig. 3 illustrates the effect of the removal of Ca^{2+} at two glucose concentrations on the inter-conversion of phosphorylase, glycogen synthase and pyruvate kinase. It is apparent that the activation of phosphorylase by phenylephrine was greatly diminished both by a high concentration of glucose and by the removal of Ca^{2+} and was abolished when these two conditions were combined. Glycogen synthase was inactivated by phenylephrine with and without Ca^{2+} , whereas, as described above, pyruvate kinase became inactivated only in the absence of Ca^{2+} . Glucose concentration had little effect on this inactivation.

Discussion

Control of gluconeogenesis

The slight stimulation of gluconeogenesis by phenylephrine and vasopressin reported in the present paper is in good agreement with previous observations by others (Hems & Whittton, 1973; Tolbert *et al.*, 1973; Exton & Harper, 1975). The fact that the phenylephrine effect was counteracted by insulin is an original observation to be put in relation with a similar antagonistic effect on the activation of phosphorylase (van de Werve *et al.*, 1977a).

The main problem that we wish to discuss is the molecular mechanism by which phenylephrine and vasopressin stimulate gluconeogenesis.

Activity of pyruvate kinase. Three different approaches have been used to answer the question of whether the effect of phenylephrine and vasopressin on gluconeogenesis is related to a change in the activity of pyruvate kinase.

(1) The activity of pyruvate kinase measured at physiological concentration of phosphoenolpyruvate (0.13 mM) was not changed by the addition of phenylephrine or vasopressin, indicating that the enzyme was not inactivated by phosphorylation. Accordingly, phenylephrine and vasopressin did not change the activity of cyclic AMP-dependent protein kinase measured with histones as substrate.

(2) The concentration of phosphoenolpyruvate was not significantly increased after phenylephrine. As discussed by Feliu *et al.* (1976), the concentration of this metabolite is expected to be elevated when pyruvate kinase is inactive. Indeed, the steady-state concentration of phosphoenolpyruvate in the cell results from a balance between an input via phosphoenolpyruvate carboxykinase and an output via enolase and pyruvate kinase. Since the concentration of phosphoenolpyruvate was not affected by phenylephrine, we can reasonably assume that the activity of pyruvate kinase was unchanged. However, we

Table 3. Influence of phenylephrine and glucagon on the rate of conversion of fructose into glucose and lactate in hepatocytes isolated from fed rats and incubated in the presence of 2.5 mM- Ca^{2+}

Hepatocytes were incubated for 15 min with 1 mM-[U- ^{14}C]fructose. Values shown are means $\pm$ S.E.M. for three observations with one batch of cells.

Addition	Conversion ($\mu\text{mol}/15 \text{ min per g of cells}$) of:	
	Fructose into glucose	Fructose into lactate
0.15 M-NaCl	4.05 $\pm$ 0.2	5.23 $\pm$ 0.5
10 μM -Phenylephrine	6.33 $\pm$ 0.4	3.65 $\pm$ 0.3
1 μM -Glucagon	9.15 $\pm$ 0.3	0.97 $\pm$ 0.03

cannot reject the possibility that a change in activity of pyruvate kinase would be counteracted by a similar change in activity of phosphoenolpyruvate carboxykinase. It has indeed been shown that adrenaline, but not glucagon, slightly increased the pyruvate kinase flux (Rognstad & Katz, 1977). As the concentration of phosphoenolpyruvate is not increased, it is unlikely that these postulated changes in enzyme activities could cause an increased production of glucose.

(3) Accordingly, the release of ^3H from [6- ^3H]glucose was not diminished by phenylephrine or vasopressin, but on the contrary it was slightly, but significantly, increased. The use of [6- ^3H]glucose allows an estimation of the glycolytic flux (Katz & Rognstad, 1975), and our results suggest that neither pyruvate kinase nor phosphofructokinase activity is decreased. These data are in sharp contrast with the effect of glucagon, which inactivated pyruvate kinase, increased the concentration of phosphoenolpyruvate and markedly decreased the release of ^3H from [6- ^3H]glucose.

We therefore conclude that the stimulation of gluconeogenesis by phenylephrine and vasopressin that is observed in the presence of Ca^{2+} does not result from a decreased activity of pyruvate kinase. Consequently there is no need to postulate an inhibition of the enzyme activity by an effector or an inactivation of the enzyme by phosphorylation that would be caused by a protein kinase dependent or not on cyclic AMP.

The fact that phenylephrine stimulates gluconeogenesis not only from pyruvate but also from fructose at the expense of its conversion into lactate could result from an activation of a metabolic step beyond the triose phosphates. An action at the level of fructose biphosphatase is a possible mechanism; an activation of this step would, however, decrease the concentration of phosphoenolpyruvate by a pull mechanism unless the activity of phosphoenolpyruvate carboxykinase is increased.

Role of Ca^{2+} . In the absence of Ca^{2+} , phenylephrine, but not vasopressin, caused an activation of histone kinase accompanied by an inactivation of pyruvate kinase. Simultaneously the effects of vasopressin on

both phosphorylase activation and gluconeogenesis were completely abolished, whereas that of phenylephrine persisted. Since the effect of phenylephrine is completely different in hepatocytes incubated in the absence of Ca^{2+} it is not possible to answer the question whether the phenylephrine effect is dependent on the presence of Ca^{2+} . By analogy with vasopressin, one could assume that the effect of phenylephrine and vasopressin in the presence of Ca^{2+} may be mediated by this cation.

Although our data are in agreement with the finding of Chan & Exton (1977) that phenylephrine caused an accumulation of cyclic AMP in hepatocytes incubated in the absence of Ca^{2+} , they are, however, in contradiction with the previous report of the same group (Assimakopoulos-Jeannet *et al.*, 1977) showing that in Ca^{2+} -depleted hepatocytes phenylephrine did not change the activity of histone kinase.

Effect of phenylephrine on the enzymes of glycogen metabolism

Several of our observations in this field confirm previous ones. It has indeed been shown that, in hepatocytes incubated with Ca^{2+} , phenylephrine and vasopressin caused an activation of phosphorylase, an inactivation of glycogen synthase with no change in phosphorylase kinase and cyclic AMP-dependent histone kinase (Hutson *et al.*, 1976; Assimakopoulos-Jeannet *et al.*, 1977; Keppens *et al.*, 1977; van de Werve *et al.*, 1977a). As already suggested (Hutson *et al.*, 1976; Keppens & De Wulf, 1977), the inactivation of glycogen synthase might result from the known inhibition of synthase phosphatase by phosphorylase (for reviews, see Hers, 1976; Stalmans, 1976).

The antagonistic effect of insulin against the activation of phosphorylase by phenylephrine has been reported by van de Werve *et al.* (1977a); the effect reported in Table 1, although smaller, confirms this previous observation.

In the absence of Ca^{2+} and with a high concentration of glucose the activation of phosphorylase by phenylephrine was completely cancelled. The inactivation of synthase, however, still took place. This

effect is presumably secondary to the activation of the cyclic AMP-dependent protein kinase that is observed in these conditions.

It is noteworthy that a high glucose concentration greatly interferes with the activation of phosphorylase, with no effect on the inactivation of pyruvate kinase. This effect of glucose on phosphorylase is easily explained by the stimulatory effect that it exerts on phosphorylase phosphatase (Stalmans, 1976).

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References

- Assimacopoulos-Jeannet, F. D., Blackmore, P. F. & Exton, J. H. (1977) *J. Biol. Chem.* **252**, 2662–2669
- Blair, J. B., Cimbala, M. A., Foster, J. L. & Morgan, R. A. (1976) *J. Biol. Chem.* **251**, 3756–3762
- Bontemps, F., Hue, L. & Hers, H. G. (1978) *Biochem. J.* **174**, 603–611
- Chan, T. M. & Exton, J. H. (1977) *J. Biol. Chem.* **252**, 8645–8651
- Cherrington, A. D., Assimacopoulos, F. D., Harper, S. C., Corbin, J. D., Park, R. C. & Exton, J. H. (1976) *J. Biol. Chem.* **251**, 5209–5218
- Czok, R. & Lamprecht, W. (1974) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), 2nd edn., vol. 3, pp. 1446–1451, Academic Press, New York
- Exton, J. H. & Harper, S. C. (1975) *Adv. Cyclic Nucleotide Res.* **5**, 519–532
- Feliu, J. E., Hue, L. & Hers, H. G. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2762–2766
- Feliu, J. E., Hue, L. & Hers, H. G. (1977) *Eur. J. Biochem.* **81**, 609–617
- Hems, D. A. (1977) *FEBS Lett.* **80**, 237–245
- Hems, D. A. & Whitton, P. D. (1973) *Biochem. J.* **136**, 705–710
- Hers, H. G. (1976) *Annu. Rev. Biochem.* **45**, 167–189
- Hue, L. & Feliu, J. E. (1978) *Biochem. Soc. Trans.* **6**, 29–33
- Hue, L., Bontemps, F. & Hers, H. G. (1975) *Biochem. J.* **152**, 105–114
- Hutson, N. J., Brumley, F. T., Assimacopoulos, F. D., Harper, S. C. & Exton, J. H. (1976) *J. Biol. Chem.* **251**, 5200–5208
- Katz, J. & Rognstad, R. (1975) *Curr. Top. Cell. Regul.* **10**, 237–289
- Keppens, S. & De Wulf, H. (1975) *FEBS Lett.* **51**, 29–32
- Keppens, S. & De Wulf, H. (1977) *Abstr. FEBS Meet. 11th* abstr. no. A 1–9 107
- Keppens, S., Van Den Heede, J. R. & De Wulf, H. (1977) *Biochim. Biophys. Acta* **496**, 448–457
- Kirk, C. J. & Hems, D. A. (1974) *FEBS Lett.* **47**, 128–131
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
- Krebs, H. A., Cornell, N. W., Lund, P. & Hems, R. (1974) *Alfred Benzon Symp.* **6**, 718–743
- Ljungström, O., Hjelmlquist, G. & Engström, L. (1974) *Biochim. Biophys. Acta* **358**, 289–298
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Patterson, M. S. & Greene, R. C. (1965) *Anal. Chem.* **37**, 854–857
- Pilkis, S. J., Claus, T. H., Johnson, R. A. & Park, C. R. (1975) *J. Biol. Chem.* **250**, 6328–6336
- Riou, J. P., Claus, T. H. & Pilkis, S. J. (1976) *Biochem. Biophys. Res. Commun.* **73**, 591–599
- Rognstad, R. & Katz, J. (1977) *J. Biol. Chem.* **252**, 1831–1833
- Sherline, P., Lynch, A. & Glinsmann, W. H. (1972) *Endocrinology* **91**, 680–690
- Stalmans, W. (1976) *Curr. Top. Cell. Regul.* **11**, 51–97
- Stubbs, M., Kirk, C. J. & Hems, D. A. (1976) *FEBS Lett.* **69**, 199–202
- Tolbert, M. E. M., Butcher, F. R. & Fain, J. N. (1973) *J. Biol. Chem.* **248**, 5686–5692
- Van Berkel, T. J. C., Kruijt, J. K., Koster, J. F. & Hülsmann, W. C. (1976) *Biochem. Biophys. Res. Commun.* **72**, 917–925
- van de Werve, G., Hue, L. & Hers, H. G. (1977a) *Biochem. J.* **162**, 135–142
- van de Werve, G., Stalmans, W. & Hers, H. G. (1977b) *Biochem. J.* **162**, 143–146
- Walsh, D. A., Ashby, C. D., Gonzalez, C., Calkins, D., Fischer, E. H. & Krebs, E. G. (1971) *J. Biol. Chem.* **246**, 1977–1985